

Hydrogels with Prestressed Tensegrity Structures

Corresponding Author: Professor Yi Cao

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

Summary :

In this manuscript, the authors use tensegrity inspired by biological systems such as extracellular matrix, as a framework for building tough hydrogels with excellent mechanical properties and water retention. Amino acid based crystal precursors are added to a preformed hydrogel, and crystal growth was triggered using an enzyme. The results indicate that the crystal growth pre-stresses the polymer network, distributes stresses and improves the mechanical properties. The approach is novel and the enzyme-induced crystal growth bridges the gap between synthetic materials and biological design principles. The manuscript is well written and the methods are detailed.

Major comments :

1. Line 72 – 76 state “However, these methods generally result in polymeric materials that display asymmetric mechanical responses along different axes of applied forces, rather than bimodulus or nonreciprocal mechanical responses—different behaviors under forces applied in opposite directions along the same axis”.

It is still unclear how “bimodulus or nonreciprocal mechanical response” is particularly useful for practical applications of hydrogels, despite observing such properties in biological tensegrity systems. Can a soft polymer dispersed with stiff rod like fillers or a double network hydrogel also show non-reciprocal mechanical response, due to one network being stressed by the other? Elaborating why

“nonreciprocal mechanical responses” is useful for applications can benefit the reader.

2. Line 205-207, “To reliably measure changes in GFP fluorescence and eliminate the impact of decreased transmittance during crystal growth, we dispersed microspheres with stable red fluorescence into the hydrogel as a reference”.

Could the authors explain how this eliminates the decreased transmittance due to opacity? ALP/PAAm hydrogels are transparent, so both the green and red fluorescence markers can be observed clearly. In the TG hydrogels, crystal growth makes the hydrogel opaque. Hence the intensity of both red and green fluorescence markers can equally decline. Also for some reason, the green fluorescent microspheres appear more visible in TG hydrogels (Supplementary figure 13) compared to the red microspheres used in Supplementary figure 9, after few hours. Are the red microspheres the best choice, compared to any other mechanochemical techniques?

3. Line 215-217 “The equivalent tensile force at each crosslinking site (FT) in TG hydrogels was calculated based on hydrogel crosslinking density (Fig. 3e)”.
Supplementary figure 11 shows that the ALP/PAAm hydrogel is stretched under

tension and the corresponding change in fluorescence is correlated to the applied stress. This stress was used to calculate the equivalent forces at each crosslinking point. There can be potential caveats to this calculation, a) In the case of the TG hydrogels with crystals, the mechanical properties of the crystal can result in a different stress field under tension and complicate the force calculation. b) The applied stress can come from entanglements or crystal polymer interactions. Therefore, the authors should state the potential errors in such analysis.

4. Supplementary figure 19 shows the difference in mechanical properties after dissolving the crystals. What do the authors expect, if the crystals de-crystallise or break into fragments under gentle heating or cyclic loading? Can the breaking of the crystals permanently damage the networks or lead to loss of tensegrity? Will the recrystallisation recover the properties?

5. Although mixing the crystals with the polymer network precursors did not result in a tensegrity structure, can this technique somehow be extended to dry elastomers? Say a soft polymer network is filled with a precursor of a stiff rigid anisotropic polymer. If the crosslinking of the stiff polymer is triggered inside the soft polymer network under stretching, can the tension generated by the stiff polymer lead to tensegrity?

Minor comments :

1. Page 8, Line 160 – 163, “Additionally, TG hydrogels exhibited a much rougher surface at the microscopic level after crystal growth, suggesting that the polymer network was locally stretched by the grown crystal sticks, similar to the irregular shape of living cells with a tensegrity structure”. A reference to such roughness observations in living cells would be interesting. Could the observed surface roughness be attributed to factors other than crystal growth? Do the hydrogels simply mixed with crystals also show no roughness?

2. Line 172 – 174 “Remarkably, the TG hydrogel's pore size (Fig. 2h) was larger than that of the ALP/PAAm hydrogel”. Could the authors elaborate why this is remarkable? Is it due to the crystals applying tension and stretching the network and pores?

3. Figure 2k misses scale value on the scale bar.

4. Lines 177-179 “TG hydrogels could be compressed to nearly 100% strain (Fig. 2i) and extended to more than 200% of their original length (Fig. 2j)”. The authors can replace “200% of their original length” with “100% tensile strain”, just to be consistent with compressive strain.

5. Caption of Supplementary Figure 9 can say Images of “TG Hydrogel”, instead of “same hydrogel” for clarity.

6. Can a standard technique like DIC be used to confirm the long term stress distribution of the gels, compared to using fluorescent probes?

Recommendation :

Publish after Minor revisions.

Reviewer #2

(Remarks to the Author)

The article describes a novel procedure to improve the stiffness of a hydrogel by immobilizing tyrosine seed crystals and alkaline phosphatase (ALP) in a hydrogel leaving this in a solution containing tyrosine phosphate. The tyrosine phosphate is cleaved to by ALP to hardly soluble tyrosine, which is then inducing growth of the crystal seeds. The resulting hydrogels show improved stiffness and compression strength while having a water content of more than 80 wt%. Additional to this not so impressing mechanical properties, the authors claim that crystal growth can be mechanically controlled, which would enable anisotropic mechanical properties. This would indeed make the material superior to existing ones. The present manuscript is a very busy work with many analytical and theoretical considerations, but no experimental proof of the proposed materials concept. There is no proof that the material can be imprinted with anisotropic mechanical properties by applying external stress. Therefore, these claims cannot be held up and this weakens the manuscript.

Modelling is useless without experimental proof. The tensegrity structures should be resulting in an anisotropic stiffness as is the case for bones. Thus, all the theoretical statements and fluorescence probes do not show this anisotropy. Further, none of the freeze-dried samples shows any anisotropic growth as always stated in the manuscript. The orienting of needle crystals in hydrogels at extreme mechanical deformation is expected and well known, but does not require any directed crystal growth and works with any hydrogel system. At the current state, the manuscript is reporting on a novel concept to use the known ALP-method with an extremely expensive system to obtain hydrogels that are not particularly well performing compared to other stiff hydrogels.

There are - besides the typos - some other points that should be addressed:

What is the crystal density? I do not understand the unit. Density is something like g/cm³ or vol/vol, but never a 2D unit. It should be provided, what the volume or the weight content of the tyrosine crystals is within the matrix.

Ratio E_t/E_c is pointless, because the compression modulus is varying along the curve.

What are the values of the fracture strain of the notched samples and how does such a curve look?

Figure 4 is misleading, because the authors have used arbitrary literature to make their material look exceptional. There are mineralized hydrogels that have more than 300 MPa tensile stiffness, more than 70 wt% water and more than 2000 J/m² and very high tensile strength. There should be a thorough literature study for this figure.

The manuscript is very difficult to read, because major data are in the supplements and thus the manuscript cannot be understood without frequently flipping to the SI. It would be better to reduce the non-proven over claims (like the cartoons) and present this study as full paper containing all necessary data in the main text.

Reviewer #3

(Remarks to the Author)

This study presents a novel approach for the fabrication of a tensegrity-structured hydrogel through the induction of amino acid crystal. The authors demonstrate that this method exhibits a certain degree of versatility and can be applied to other hydrogel systems. This work offers a breakthrough solution for the elusive combination of high stiffness, strength, water content, and bimodal mechanical response. This innovation has the potential to revolutionize the field of biomimetic materials and may serve as a classic example in the fabrication of gel materials. The manuscript is well-structured, with clear writing, comprehensive data presentation, and high-quality graphical content. It deserves publication in this journal. Other specific comments and suggestions are as follows:

1.Challenges of method scalability and technical barriers. The designed hydrogel materials exhibit remarkable properties, including high water content, stiffness, and toughness. However, the preparation method is relatively complex, particularly the bio-synthesis step, which requires long processing times and involves a high technical barrier. The potential for broader adoption of this interdisciplinary approach, or how to engage a wider community of hydrogel researchers, is an issue worth considering. The authors might contemplate discussing the scalability of this method, exploring how it could be simplified or made more accessible to a broader audience, especially considering the involvement of bio-synthesis.

2.Comprehensive evaluation of the strengths and weaknesses of gel mechanical properties. Compared to glassy-state hydrogels (Chem. Mater. 2019, 31 (4)); Chem. Eng. J. 2023. 457 (141280)) the hydrogel presented in this study exhibits notable advantages, including high water content (>80%) and a bimodal mechanical response. In the Introduction or Discussion sections, the authors should provide further explanations of the significance and potential applications of these two key advantages. A clearer rationale for why these properties are desirable, along with specific contexts in which these hydrogels could be applied, would strengthen the application relevance of the study.

3.Additional evidence of key role of the prestressed polymer matrix at the bulk level. As discussed throughout the manuscript, the prestressed polymer matrix plays a critical role in the tensegrity structures and contributes to the excellent mechanical properties. While the tension force on the polymers at the nano/microscale has been thoroughly evaluated, it would be beneficial to provide additional evidence for the role of the tensioned polymer network at the bulk level, such as the release of tension force through moderate dehydration.

4.Further processing of the tensegrity structures hydrogels. As described in the manuscript, the tensegrity structure was fabricated through in-situ crystal growth. Since the ALP enzyme is covalently linked to the hydrogel network, it would be valuable to explore methods for spatially confining the tensegrity structures within the hydrogel. This could potentially serve as an additional processing technique for these hydrogels.

5.Some minor issues. (1)According to the figure S2, the group of “-NH-” at the left terminal is labeled in red. As I am concerned, it might come from the carboxyl group form alanine next to acrylate, in which case it should be labeled in black. (2)It's recommended to include a flow chart for the preparation process of hydrogels to help with the quick understanding of the formation of tensegrity structured hydrogels.

Reviewer #4

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors have adequately addressed all our questions.

(Remarks on code availability)

Reviewer #2

(Remarks to the Author)

The authors have clarified to manuscript according to the reviewer suggestions and the manuscript should be published after some minor additions. Tensegrity is a structural concept with a rather unclear concept for applications. The authors should give some important examples of biotensegrity in nature and also some application possibilities, where this mechanical behaviour is of importance.

The samples clearly show plastic deformation indicated by the stress-strain-curve measured with a tensile tester. These results can hardly be compared to measurements with a dynamic mechanical analysis, which is often used for such tests. Thus, the samples are not more elastic nor stretchable than comparable materials in literature. However, the strong toughness of the notched sample should be highlighted, because this is a rare feature in stiff hydrogels.

On a minor note, the enzyme used for comparable mineralized hydrogels in supplementary Table 1 is not urea, but urease.

(Remarks on code availability)

Reviewer #3

(Remarks to the Author)

The authors have well addressed my concerns. Now the manuscript is suitable for publication in Nature Communications. An acceptance is recommended.

(Remarks on code availability)

Reviewer #4

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

(Remarks on code availability)

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Point-by-point response to the reviewers' comments

Reviewer #1 (Remarks to the Author):

Summary:

In this manuscript, the authors use tensegrity inspired by biological systems such as extracellular matrix, as a framework for building tough hydrogels with excellent mechanical properties and water retention. Amino acid based crystal precursors are added to a preformed hydrogel, and crystal growth was triggered using an enzyme. The results indicate that the crystal growth pre-stresses the polymer network, distributes stresses and improves the mechanical properties. The approach is novel and the enzyme-induced crystal growth bridges the gap between synthetic materials and biological design principles. The manuscript is well written and the methods are detailed.

General response:

We thank the reviewer for the positive comments. In order to address her/his concerns, we have conducted a substantial revision with new data, analyses and discussions. The detailed point-by-point response to each of her/his concerns is attached below. The changes to the original submission are highlighted in blue in the revised manuscript and Supplementary Information.

Major comments:

1. Line 72 – 76 state “However, these methods generally result in polymeric materials that display asymmetric mechanical responses along different axes of applied forces, rather than bimodulus or nonreciprocal mechanical responses—different behaviors under forces applied in opposite directions along the same axis”. It is still unclear how “bimodulus or nonreciprocal mechanical response” is particularly useful for practical applications of hydrogels, despite observing such properties in biological tensegrity systems. Can a soft polymer dispersed with stiff rod like fillers or a double network hydrogel also show non-reciprocal mechanical response, due to one network being stressed by the other? Elaborating why “nonreciprocal mechanical responses” is useful

for applications can benefit the reader.

Response:

We thank the reviewer for the comments. We anticipate that the bimodulus or nonreciprocal mechanical responses observed in TG hydrogels could have significant applications in tissue engineering. For example, in cells, the tensegrity structure supports the resilience and adaptability of the cytoskeleton and extracellular matrix (J. Cell Sci. 1993, 104 (Pt 3), 613-627; Osteoarthr. Cartilage 1999, 7, 81-94; Prog. Biophys. Mol. Biol. 2008, 97, 163-179; Cureus 2020, 12, e7895), both of which exhibit nonreciprocal mechanical responses crucial for cellular functions. The tensegrity strategy presented in this work offers a general approach for fabricating bimodular synthetic hydrogels, which will undoubtedly benefit the development and application of biomaterials that more closely mimic the mechanical properties of cytoskeleton and extracellular matrix. The new discussions have been included in the revised manuscript. (See line 50-57 on Page 3 in the revised manuscript)

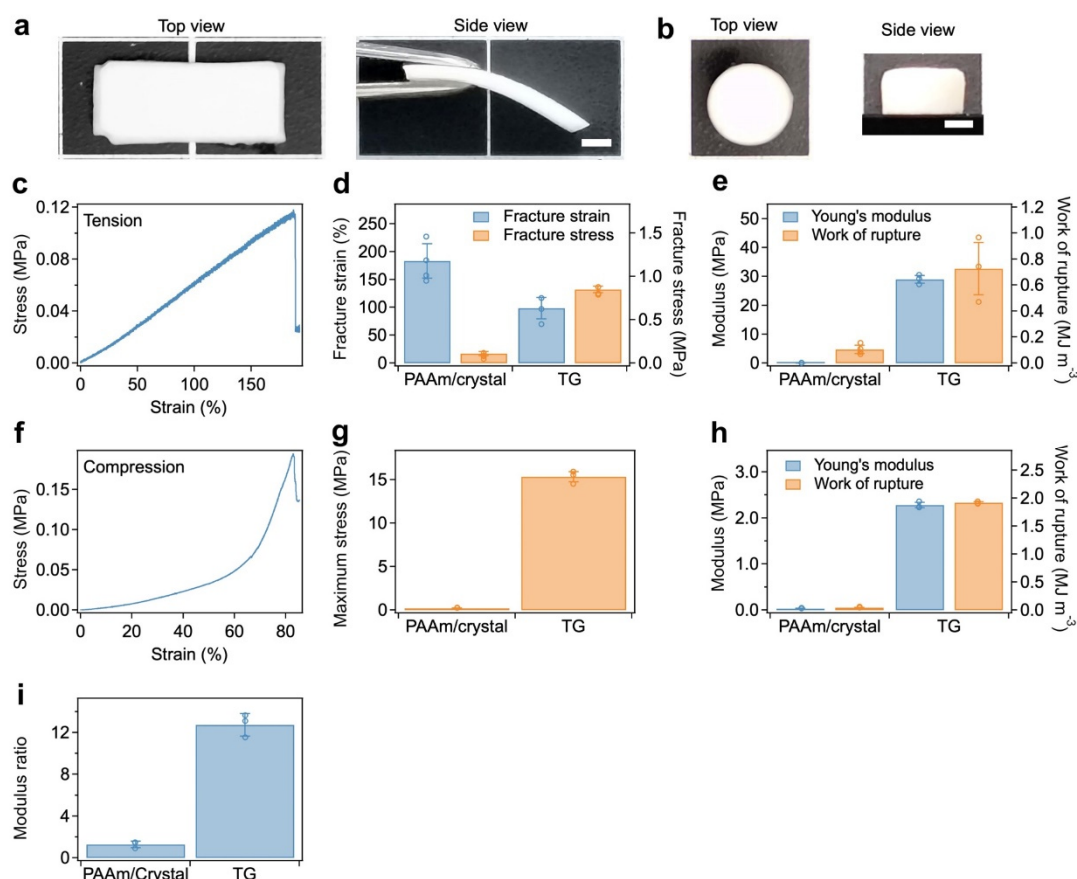
On the other hand, we expect that a soft polymer dispersed with stiff rod-like fillers or a double-network hydrogel may not exhibit a significant nonreciprocal mechanical response, as the prestress in the network is not sufficiently enhanced. In the case of a soft polymer with stiff rod-like fillers, the polymer network cannot be effectively stretched by the fillers due to the lack of in-situ filler growth. As shown in Supplementary Fig. 20 (now updated to Supplementary Fig. 21), the tensile-to-compressive modulus ratio of hydrogels prepared by simply mixing preformed tyrosine crystal sticks into PAAm hydrogels was approximately 1.3, which is significantly lower than the ~13 ratio observed in TG hydrogels. As previously reported by Gong et al. (Fig. R1, adapted from Macromolecules 2016, 49, 5, 1865–1872), the stress-stretch ratio curves by tensile test and compression test for a typical double-network hydrogel almost overlapped with each other, indicating that tension and compression give the same modulus in double-network hydrogel. Noting that the compression deformation is converted to biaxial tensile deformation for quantitative comparison in this work. These new comments have been incorporated into the revised manuscript. (See line

290-293 on Page 15-16 in the revised manuscript; See Supplementary Figure 21 on Page 18-19 in the revised Supplementary Information)

Revision:

“These unique properties have a substantial impact on the mechanical behavior of biological systems. For example, in cells, the tensegrity structure supports the resilience and adaptability of the cytoskeleton¹⁸⁻²⁰ and extracellular matrix^{21,22}, both of which exhibit nonreciprocal mechanical responses crucial for cellular functions. Hence, bio-inspired design of hydrogels incorporating tensegrity structures is a promising strategy for creating robust, adaptive materials that maintaining a high water content, which are critical for biological functions and biomimetic applications, such as tissue engineering.²³⁻²⁷”

“Simply mixing preformed tyrosine crystal sticks into the PAAm hydrogels only slightly increased their Young’s moduli and strength with a tensile-to-compressive modulus ratio less than 1.3, underscoring the importance of in-situ crystal growth for tensegrity structure formation (Supplementary Figs. 21-22).”



Supplementary Figure 21. Comparison of hydrogels prepared by mixing tyrosine crystal sticks (100 mg mL^{-1}) with precursors during preparation (PAAm/crystal hydrogels, $C_{\text{bis-acrylamide}} = 20 \text{ mM}$, $C_{\text{crystal}} = 100 \text{ mg mL}^{-1}$ and $C_{\text{SC-ALP}} = 3 \text{ mg mL}^{-1}$) and TG hydrogels ($C_{\text{bis-acrylamide}} = 20 \text{ mM}$, $C_{\text{nucleus}} = 2 \text{ mg mL}^{-1}$ and $C_{\text{SC-ALP}} = 3 \text{ mg mL}^{-1}$). **a**, **b**, Optical images of the band-shaped (**a**) and columnar (**b**) PAAm/crystal hydrogels. Scale bar = 2.5 mm. **c**, Typical stress-strain curve of the band-shaped PAAm/crystal hydrogel under tension. **d**, Summarized fracture strain and fracture stress of PAAm/crystal and TG hydrogels under tension. **e**, Summarized Young's modulus and work of rupture of PAAm/crystal and TG hydrogels under tension. **f**, Typical stress-strain curve of the columnar PAAm/crystal hydrogel under compression. **g**, Summarized maximum stress of PAAm/crystal and TG hydrogels under compression. **h**, Summarized Young's modulus and work of rupture of PAAm/crystal and TG hydrogels under compression. **i**, Ratios of tensile to compressive moduli for PAAm/crystal and TG hydrogels. PAAm/crystal hydrogels show a ratio of ~ 1.3 while TG hydrogels have a ratio of 13. Values in **d**, **e**, **g**, **h** and **i** represent the mean and

standard deviation ($n = 3$ or 4).

[REDACTED]

Figure R1. Normalized stress–deformation ratio curves, both uniaxial and equal biaxial stretching, of the St-TPEG/PAAm gels with various deformation ratio (α). (Reproduced from *Macromolecules* 2016, 49, 5, 1865–1872)

2. Line 205-207, “To reliably measure changes in GFP fluorescence and eliminate the impact of decreased transmittance during crystal growth, we dispersed microspheres with stable red fluorescence into the hydrogel as a reference”. Could the authors explain how this eliminates the decreased transmittance due to opacity? ALP/PAAm hydrogels are transparent, so both the green and red fluorescence markers can be observed clearly. In the TG hydrogels, crystal growth makes the hydrogel opaque. Hence the intensity of both red and green fluorescence markers can equally decline. Also for some reason, the green fluorescent microspheres appear more visible in TG hydrogels (Supplementary figure 13) compared to the red microspheres used in Supplementary figure 9, after few hours. Are the red microspheres the best choice, compared to any other mechanochemical techniques?

Response:

We thank the reviewer for the comments. As the reviewer noted, both the intensity of GFP fluorescence (green fluorescence markers) and microsphere fluorescence (red fluorescence markers) can decline due to the opacity of TG hydrogels. To address this, we used the ratio of GFP to microsphere fluorescence (GFP/FluoSpheres ratio) as an indicator of GFP unfolding. When the TG hydrogel transitions from a transparent to an opaque state, the GFP/FluoSpheres ratio should remain consistent because both the

green and red fluorescence intensities would decline equally. However, as crystal growth increases the tensile force within the polymer network, parts of the GFP in the hydrogel unfold, resulting in a faster decline in GFP fluorescence. Consequently, the GFP/FluoSpheres ratio decreases with the progression of crystal growth. Therefore, we used the GFP/FluoSpheres ratio as a proxy for internal stress, compensating for the reduced transmittance due to the opacity. The new comments have been added the revised manuscript. (See line 209-215 on Page 11 in the revised manuscript)

While green fluorescent microspheres would generally be a better choice due to their higher visibility, we opted for red fluorescent microspheres to avoid overlap with GFP's emission spectrum. Compared to other mechanochemical techniques, our method may not be the optimal choice because it requires stable fluorescence from both markers. This method was selected because it was feasible with the equipment available in our laboratory. Alternative mechanochemical techniques could also be employed, such as detecting released ions from coordination structures (Nat. Chem. 2021, 13, 56) or trapping free radicals with prefluorescent probes (J. Am. Chem. Soc. 2023, 145(13), 7376–7389) in polymer networks under deformation. However, it is worth noting that since the unfolding force of GFP has been well studied and reported (PNAS, 2017, 114, 11052-11056), using GFP can help us to determine the order of tension applied on the polymer. The new discussions have been included in the revised manuscript. (See line 220-222 on Page 12 and line 623-624 on Page 32 in the revised manuscript)

Revision:

“In principle, the ratio of GFP to microsphere fluorescence (GFP/FluoSpheres ratio) should remain constant, as both the GFP and microsphere fluorescence would decline equally due to the reduced transmittance of the hydrogel. However, as mentioned earlier, the unfolding of GFP would lead to a faster decline in its fluorescence compared to the microspheres. This resulted in a decrease in the GFP/FluoSpheres ratio. Therefore, the reduction in the GFP/FluoSpheres ratio served as an indicator of GFP unfolding.”

“Given that the unfolding force of GFP is reported to be in the range of 30–60 pN⁶², we anticipate that the tensile force applied to the polymer in TG hydrogels may exceed this range.”

“The microsphere with red fluorescence was chosen to avoid the overlapping of emission spectrum with GFP.”

3. Line 215-217 “The equivalent tensile force at each crosslinking site (F_T) in TG hydrogels was calculated based on hydrogel crosslinking density (Fig. 3e)”. Supplementary figure 11 shows that the ALP/PAAm hydrogel is stretched under tension and the corresponding change in fluorescence is correlated to the applied stress. This stress was used to calculate the equivalent forces at each crosslinking point. There can be potential caveats to this calculation, a) In the case of the TG hydrogels with crystals, the mechanical properties of the crystal can result in a different stress field under tension and complicate the force calculation. b) The applied stress can come from entanglements or crystal polymer interactions. Therefore, the authors should state the potential errors in such analysis.

Response:

We thank the reviewer for the comments. We fully agree with the reviewer that estimating the tensile force on each polymer chain from macroscopic stress is a bold assumption, as the force distribution among individual chains is not uniform. There may be potential caveats in the calculation of the equivalent tensile force at each crosslinking site (F_T). a) Firstly, to achieve a tensegrity structure within the hydrogels, rigid crystal sticks are necessary to mechanically interlock the network, thereby exerting significant tension on the elastic network. The localized stress fields generated by this tension may have disordered orientations, leading to heterogeneous decreases in the GFP/FluoSpheres ratios at the micrometer scale. This variation could complicate the force calculation. b) Additionally, polymer entanglements and crystal-polymer interactions within the TG hydrogels can contribute to the overall tension in the polymer network, enhancing the tensile forces on the polymers. These factors were included in

the calculation of the equivalent tensile force in this study, which may affect the calculated tensile force.

Accordingly, we have removed the discussion on quantitatively correlating macroscopic stress in hydrogels with the tensile force on polymer chains. Instead, we have added comments indicating that the decrease in the GFP/FluoSpheres ratio in TG hydrogels reflects an increase in polymer chain density under tension. Furthermore, based on the reported unfolding force of GFP (PNAS, 2017, 114, 11052–11056), the tensile force applied to the polymer is estimated to be in the range of 10–100 pN. These revisions have been incorporated into the manuscript. (See line 215-222 on Page 11-12 in the revised manuscript)

Revision:

“In TG hydrogels, the ratio decreased significantly from 70 to less than 10 within 24 hours of in-situ crystal growth (Fig. 3b-d and Supplementary Fig. 9), indicating an increase in polymer chain density under tension. In contrast, this ratio remained stable in control ALP/PAAm hydrogels without crystal growth (Fig. 3d and Supplementary Fig. 10). Given that the unfolding force of GFP is reported to be in the range of 30–60 pN⁶², we anticipate that the tensile force applied to the polymer in TG hydrogels may exceed this range.”

4. Supplementary figure 19 shows the difference in mechanical properties after dissolving the crystals. What do the authors expect, if the crystals de-crystallise or break into fragments under gentle heating or cyclic loading? Can the breaking of the crystals permanently damage the networks or lead to loss of tensegrity? Will the recrystallisation recover the properties?

Response:

We thank the reviewer for the comments. We have investigated the effects of crystal de-crystallization or fragmentation under gentle heating or cyclic loading.

First, we studied the effect of de-crystallization through gentle heating. As shown by the absence of crystals on the surface of TG hydrogels (Supplementary Fig. 18a), parts of the crystals inside the TG hydrogel de-crystallized after being heated at 70 °C for 30 minutes in a TEA solution (0.2 M, pH = 8.8), due to the increased solubility of tyrosine. This de-crystallization could potentially compromise the tensegrity within the hydrogel. As a result, the Young's modulus of the TG hydrogels decreased by approximately 20%, demonstrating the thermal stability of the TG structure (Supplementary Fig. 18b-d).

Next, we examined the effect of crystal fragmentation under cyclic loading. As shown in Supplementary Fig. 19a-b, crystals inside the TG hydrogels fragmented under cyclic loading (10 cycles of stretching-relaxation at a strain of 20% or 10 cycles of compression-relaxation at a strain of 40%). The decreased stress-strain curves indicate that the fragmentation of crystals contributed to the loss of tensegrity, leading to a reduction in Young's modulus. Moreover, the fragmented crystals were removed by dissolving them in a TEA solution (0.2 M, pH = 8.8), and the mechanical properties were re-evaluated (Supplementary Fig. 19c-d). The hydrogels with fragmented crystals removed exhibited lower Young's moduli compared to crystal-removed ALP/PAAm hydrogels (Supplementary Figs. 19e and 20c-d), suggesting that the network structure was permanently damaged to some extent. However, the Young's moduli of hydrogels with fragmented crystals removed were similar to those of crystal-removed TG hydrogels (Supplementary Fig. 20c-d), indicating that it was the in-situ crystal growth, rather than crystal fragmentation, that permanently damaged the network structure.

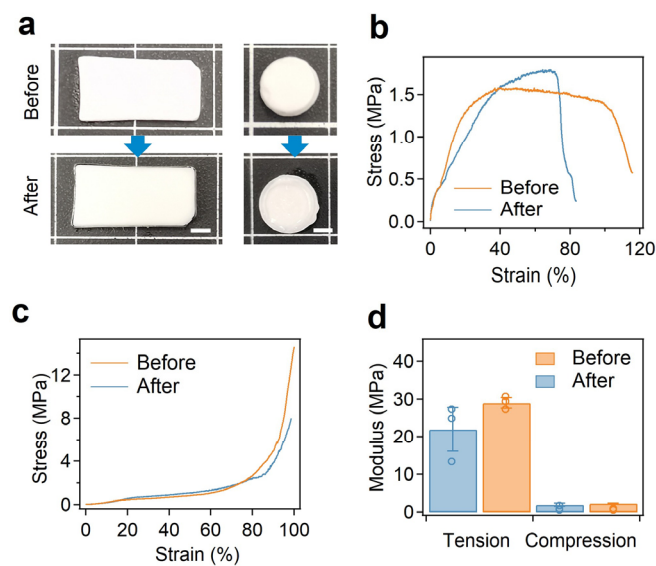
Finally, we attempted recrystallization within the hydrogels after removing the crystals. As shown in Supplementary Fig. 23a, both the TG hydrogel and the TG hydrogel subjected to cyclic loading/unloading were immersed in TEA solution (0.2 M, pH = 8.8) for 3 days to partially dissolve the crystals. The hydrogels were then incubated at 37 °C in a saturated solution of L-tyrosine (TEA buffer, 0.2 M, pH = 8.8) containing 50 mM phosphotyrosine for 2 days to achieve recrystallization, following the same preparation method as for the TG hydrogels. However, no new crystal growth was observed inside

the hydrogels, likely due to the inactivation of alkaline phosphatase (ALP) and the absence of crystal nuclei (Supplementary Fig. 23b). All these new results and relevant discussions are now incorporated into the revised manuscript. (See line 282-290 and 293-296 on Page 15-16 in the revised manuscript; See Supplementary Figures 18-19 on Page 15-17 and Supplementary Figure 23 on Page 19 in the revised Supplementary Information)

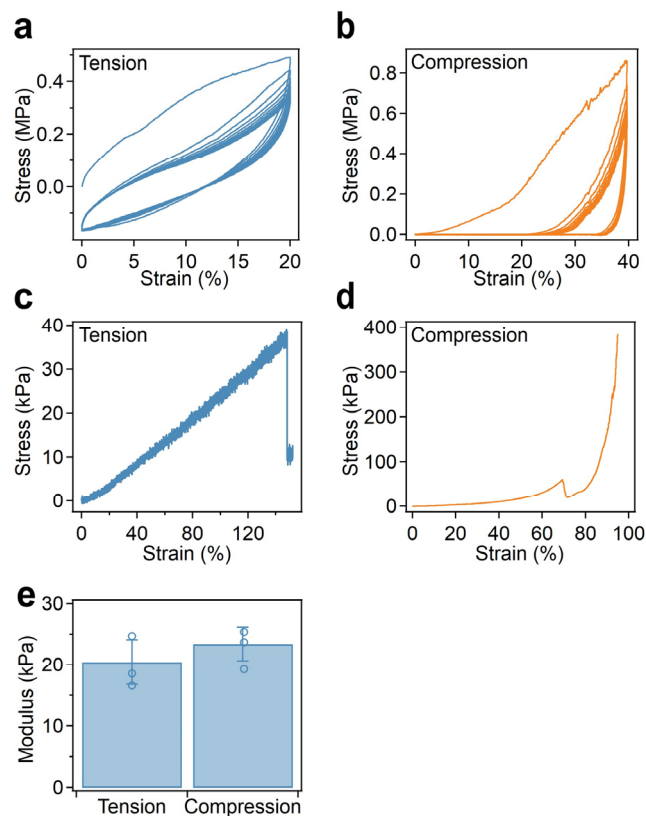
Revision:

“Partial loss of the tensegrity structures through gentle heating or cyclic loading both resulted in a decrease in Young’s modulus (Supplementary Figs. 18 and 19a-b). Complete dissolution of the rigid crystals in both TG hydrogels and TG hydrogels subjected to cyclic loading led to similarly reduced Young’s moduli of the polymer network (Supplementary Figs. 19c-e and 20), which were slightly lower than those of the original polymer network (the crystal-removed ALP/PAAm hydrogels). This suggests that in-situ crystal growth, rather than crystal fragmentation, was responsible for the slight damage to the network structure.”

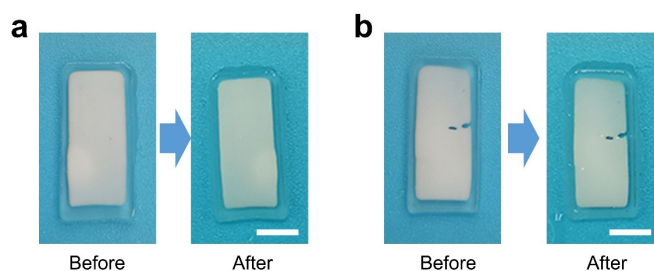
“It is noteworthy that recrystallization within the hydrogels after crystal removal could not be achieved, likely due to the inactivation of ALP and the absence of crystal nuclei (Supplementary Fig. 23).”



Supplementary Figure 18. Effects of de-crystallization through gentle heating on TG hydrogels. **a**, Optical images of band-shaped and columnar TG hydrogels before and after heating at 70 °C for 30 minutes in a TEA solution (0.2 M, pH = 8.8). **b**, **c**, Typical stress–strain curves of TG hydrogels under tension (**b**) and compression (**c**) before and after being heated at 70 °C for 30 minutes in a TEA solution (0.2 M, pH = 8.8). **d**, Summarized Young’s modulus of TG hydrogels under tension and compression before and after heating at 70 °C for 30 minutes in a TEA solution (0.2 M, pH = 8.8). Values represent the mean and standard deviation (n = 3).



Supplementary Figure 19. Effects of crystal fragmentation under cyclic loading on TG hydrogels. **a, b**, Cyclic stress–strain curves of the same TG hydrogel subjected to continuous stretching (**a**) and compressing (**b**) cycles without waiting time, at strains of 20% and 40%, respectively. **c, d**, Typical stress–strain curves of crystal-removed TG hydrogels under tension (**c**) and compression (**d**) after cyclic loading/unloading. **e**, Summarized Young's moduli of crystal removed TG hydrogels under tension and compression after cyclic loading/unloading. The tensile-to-compressive modulus ratio decreased to ~ 0.9 for crystal removed TG hydrogels after cyclic loading/unloading. Values represent the mean and standard deviation ($n = 3$).



Supplementary Figure 23. Optical images of recrystallization in different hydrogels

after partial crystal dissolution. **a**, Optical images of TG hydrogel before (left) and after (right) recrystallization for 2 days. The hydrogel was first immersed in TEA solution (0.2 M, pH 8.8) for 3 days to partially dissolve the crystals, followed by incubation at 37 °C in a saturated L-tyrosine solution (TEA buffer, 0.2 M, pH 8.8) containing 50 mM phosphotyrosine for 2 days to induce recrystallization. **b**, Optical images of TG hydrogel subjected to cyclic loading/unloading before (left) and after (right) recrystallization for 2 days. The hydrogel underwent cyclic loading/unloading and was then treated with TEA solution (0.2 M, pH 8.8) for 3 days to partially dissolve the crystals. It was subsequently incubated at 37 °C in a saturated L-tyrosine solution (TEA buffer, 0.2 M, pH 8.8) containing 50 mM phosphotyrosine for 2 days to facilitate recrystallization.

5. Although mixing the crystals with the polymer network precursors did not result in a tensegrity structure, can this technique somehow be extended to dry elastomers? Say a soft polymer network is filled with a precursor of a stiff rigid anisotropic polymer. If the crosslinking of the stiff polymer is triggered inside the soft polymer network under stretching, can the tension generated by the stiff polymer lead to tensegrity?

Response:

We thank the reviewer for the comments. We anticipate that this technique can be extended to dry elastomers. To achieve a tensegrity structure inside soft materials, several key factors need to be designed rationally. First, elements with different stiffnesses are required, where the stiff elements serve as the interlocked rigid skeleton and the soft elements function as soft linkers. Second, the stiff elements with a large aspect ratio should have a disordered orientation to facilitate their mechanical interlocking at low mass contents. Third, the soft linkers or elastic soft network must be prestressed to enable immediate stress transfer upon mechanical loading, maintaining structural integrity even under significant strain. Although the prestressing of the soft polymer network in this work was achieved through in-situ crystal growth in aqueous solutions, it can also be achieved by other methods in dry elastomers. For a soft polymer network filled with a precursor of a stiff rigid anisotropic polymer, the

tension generated by the stiff polymer can also induce tensegrity when the crosslinking of the stiff polymer is triggered inside the soft polymer network under stretching. These discussions have now been incorporated into the revised manuscript. (See line 489-503 on Page 26-27 in the revised manuscript)

Revision:

“To achieve a tensegrity structure inside soft materials, several key factors need to be designed rationally. First, elements with different stiffnesses are required, where the stiff elements serve as the interlocked rigid skeleton and the soft elements function as soft linkers. Second, the stiff elements with a large aspect ratio should have a disordered orientation to facilitate their mechanical interlocking at low mass contents. Third, the soft linkers or elastic soft network must be prestressed to enable immediate stress transfer upon mechanical loading, maintaining structural integrity even under significant strain. Following these rules, the tensegrity can also be extended to other hydrogel system. For example, by inducing the in-situ crystal growth of inorganic salt at large aspect ratios via hydrothermal process or catalytic reduction, tensegrity in hydrogels can be achieved without bio-synthesis steps. Moreover, tensegrity can also be achieved in other soft materials such as dry elastomers. For example, by introducing a soft polymer network filled with a precursor of a stiff, rigid anisotropic polymer, the tension generated by the stiff polymer can induce tensegrity when the crosslinking of the stiff polymer is triggered within the soft polymer network under stretching.”

Minor comments:

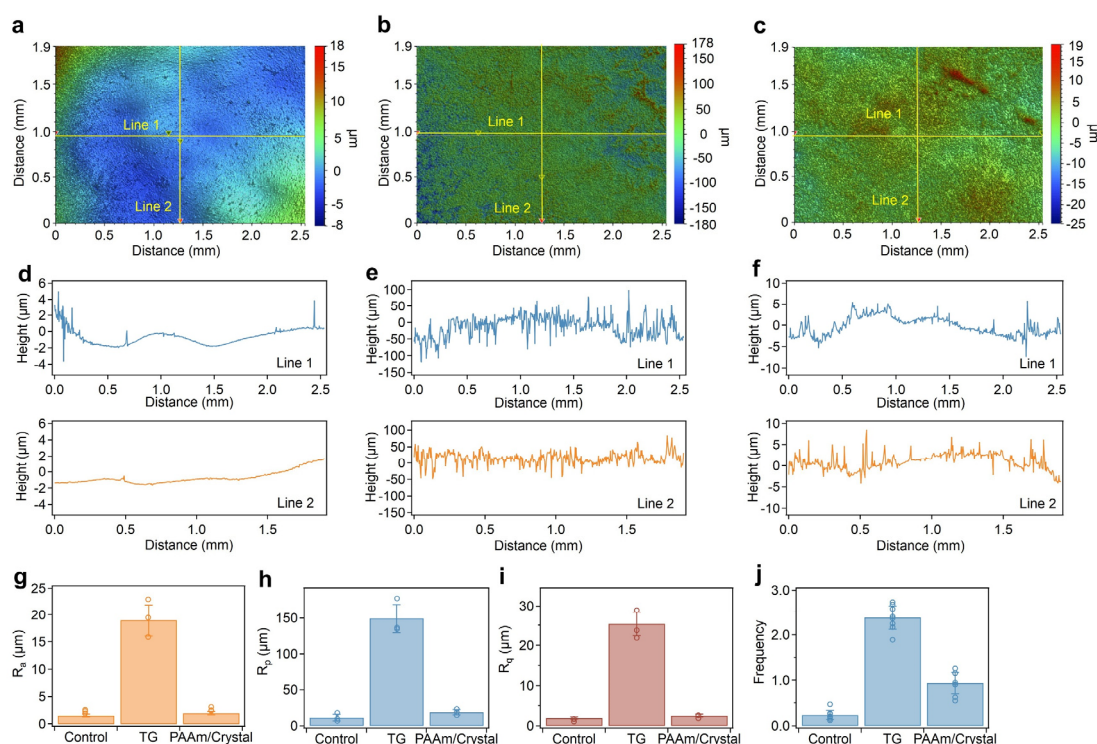
1. Page 8, Line 160 – 163, “Additionally, TG hydrogels exhibited a much rougher surface at the microscopic level after crystal growth, suggesting that the polymer network was locally stretched by the grown crystal sticks, similar to the irregular shape of living cells with a tensegrity structure”. A reference to such roughness observations in living cells would be interesting. Could the observed surface roughness be attributed to factors other than crystal growth? Do the hydrogels simply mixed with crystals also show no roughness?

Response:

We thank the reviewer for the comments. Following the reviewer's suggestion, we have cited references on roughness observations in living cells (Refs. 59-61). Additionally, to confirm whether the surface roughness is due to crystal growth or merely the mixing of crystals, we examined the surface roughness of hydrogels prepared by simply mixing crystals. As shown in the updated Supplementary Fig. 5, the surface roughness of these hydrogels was significantly smaller than that of the TG hydrogels, indicating that the enhanced roughness is primarily attributed to the crystal growth within the hydrogels. These new data and comments have been included in the revised manuscript. (See line 159-162 on Page 8 in the revised manuscript; See Supplementary Figure 5 on Page 7 in the revised Supplementary Information)

Revision:

“Additionally, TG hydrogels exhibited a much rougher surface than both ALP/PAAm hydrogels and those prepared by directly mixing tyrosine crystal sticks, suggesting that the polymer network was locally stretched by the grown crystal sticks, similar to the irregular shape of living cells with a tensegrity structure (Supplementary Fig. 5).⁵⁹⁻⁶¹”



Supplementary Figure 5. Surface roughness evaluation of ALP/PAAm hydrogels, TG hydrogels and hydrogels prepared by mixing tyrosine crystal sticks with precursors during preparation (PAAm/Crystal hydrogels). **a-c**, Typical 3D reconstruction images of the surfaces of ALP/PAAm hydrogels (**a**), TG (**b**) hydrogels and PAAm/Crystal hydrogels (**c**). **d-f**, Height profiles along the surface lines of ALP/PAAm hydrogels (**d**), TG hydrogels (**e**) and PAAm/Crystal hydrogels (**f**). **g-i**, Arithmetical mean deviation (R_a , **g**), maximum profile peak height (R_p , **h**), and root mean square deviation (R_q , **i**) for the surfaces of different hydrogels. **j**, Crystal frequency per 100 μm on the surface of different hydrogels. Values in **g-j** represent the mean and standard deviation ($n \geq 3$).

2. Line 172 – 174 “Remarkably, the TG hydrogel's pore size (Fig. 2h) was larger than that of the ALP/PAAm hydrogel”. Could the authors elaborate why this is remarkable? Is it due to the crystals applying tension and stretching the network and pores?

Response:

We thank the reviewer for the comments. As the reviewer suggested, we anticipate that the larger pore size observed in the lyophilized TG hydrogels is due to the tensional force applied by the crystals, which stretched the hydrogel network and consequently expanded the pore size. This explanation has been included in the revised manuscript. (See line 172-175 on Page 9 in the revised manuscript)

Revision:

“Remarkably, the TG hydrogel's pore size (Fig. 2h) was larger than that of the ALP/PAAm hydrogel (Fig. 2f). This can be attributed to the tensional force applied by the crystals, which stretched the hydrogel network and thereby expanded the pore size.”

3. Figure 2k misses scale value on the scale bar.

Response:

We thank the reviewer for the comments. Now we have added the missed scale value on the scale bar in the caption of Figure 2k. (See line 197 on Page 10 in the revised manuscript)

Revision:

“The scale bars in the middle images represent 5 mm.”

4. Lines 177-179 “TG hydrogels could be compressed to nearly 100% strain (Fig. 2i) and extended to more than 200% of their original length (Fig. 2j)”. The authors can replace “200% of their original length” with “100% tensile strain”, just to be consistent with compressive strain.

Response:

We thank the reviewer for the comments. Now we have revised the statement following the reviewer’s suggestion. (See line 179-180 on Page 9 in the revised manuscript)

Revision:

“TG hydrogels could be compressed to nearly 100% strain (Fig. 2i) and extended to over 120% tensile strain (Fig. 2j).”

5. Caption of Supplementary Figure 9 can say Images of “TG Hydrogel”, instead of “same hydrogel” for clarity.

Response:

We thank the reviewer for the comments. Now we have revised “same hydrogel” into “TG Hydrogel” in the caption of Supplementary Figure 9 following the reviewer’s comments. (See the caption of Supplementary Figure 9 on Page 10 in the revised Supplementary Information)

Revision:

“**Supplementary Figure 9.** Images of a **TG Hydrogel** containing SC-GFP-SC and red fluorescent microspheres after crystal growth in TEA buffer (0.2 M, pH=8.8) containing phosphotyrosine (50 mM) for different times (0.1, 0.25, 0.5, 0.75, 1, 2, 3, 6, 12 and 24 h). The grey channel indicates the GFP/FluoSpheres ratios.”

6. Can a standard technique like DIC be used to confirm the long term stress distribution of the gels, compared to using fluorescent probes?

Response:

We thank the reviewer for the comments. In principle, a standard technique like digital image correlation (DIC) could be used to confirm the long-term stress distribution of the gels, as it also provides insight into the stress field distribution. However, the displacement measurement accuracy of most commercially available DIC systems (e.g., ARAMIS 3D & HHS, GOM, Germany) exceeds 10 μm , which does not meet the sub-2 μm accuracy required for this work. Therefore, we employed a custom-designed method for detecting fluorescent microsphere movement using a confocal fluorescence microscope and image analysis (Fig. 3e-g).

Recommendation:

Publish after Minor revisions.

Response:

We thank the reviewer for the positive comments.

Reviewer #2 (Remarks to the Author):

The article describes a novel procedure to improve the stiffness of a hydrogel by immobilizing tyrosine seed crystals and alkaline phosphatase (ALP) in a hydrogel leaving this in a solution containing tyrosine phosphate. The tyrosine phosphate is cleaved to by ALP to hardly soluble tyrosine, which is then inducing growth of the crystal seeds. The resulting hydrogels show improved stiffness and compression strength while having a water content of more than 80 wt%. Additional to this not so impressing mechanical properties, the authors claim that crystal growth can be mechanically controlled, which would enable anisotropic mechanical properties. This would indeed make the material superior to existing ones. The present manuscript is a very busy work with many analytical and theoretical considerations, but no experimental proof of the proposed materials concept. There is no proof that the material can be imprinted with anisotropic mechanical properties by applying external stress. Therefore, these claims cannot be held up and this weakens the manuscript.

Response:

We thank the reviewer for his/her careful reading and valuable feedback. We would like to clarify that our work does not claim that the TG (tensegrity) hydrogels possess “anisotropic” mechanical properties in the conventional sense. Instead, our hydrogels exhibit a bimodular (or nonreciprocal) mechanical response—that is, they show different behaviors under tension versus compression along the same axis. We believe this distinction may have led to some of the reviewer’s concerns.

Below is our response to his/her comments.

"The article describes a novel procedure to improve the stiffness of a hydrogel by immobilizing tyrosine seed crystals and alkaline phosphatase (ALP) in a hydrogel leaving this in a solution containing tyrosine phosphate. The tyrosine phosphate is cleaved by ALP to hardly soluble tyrosine, which is then inducing growth of the crystal seeds. The resulting hydrogels show improved stiffness and compression strength while having a water content of more than 80 wt%."

Our Response:

We thank the reviewer for accurately summarizing the chemical procedure used in our work. While we agree that the chemistry of tyrosine phosphate dephosphorylation and subsequent crystal growth has been described in other contexts, our study uniquely demonstrates that this in-situ crystal growth in a preformed hydrogel network—leads to the formation of tensegrity structures. It is this special combination of structural design and mechanochemical interplay that results in a unique set of mechanical property (high stiffness, high compression strength, and high water content) that has not been achieved in hydrogels lacking tensegrity architectures. We have clarified this point in the revised manuscript. (See line 457-466 on Page 25 in the revised manuscript)

"Additional to this not so impressive mechanical properties, the authors claim that crystal growth can be mechanically controlled, which would enable anisotropic mechanical properties. This would indeed make the material superior to existing ones."

Our Response:

We respectfully disagree with this interpretation. Our manuscript does not claim that the crystal growth is mechanically controlled in the sense of externally imprinting anisotropy. Instead, we show that the growth of the crystal seeds intrinsically leads to the formation of tensegrity structures within the hydrogel. This internal process induces a sharp transition in mechanical properties—not because of an imposed external anisotropy, but as a natural outcome of the structure's evolution. The enhanced mechanical properties arise from this unique interplay between rigid crystal sticks and the prestressed, soft polymer network, rather than from an externally controlled directional imprinting. We have clarified this point in the revised manuscript. (See line 449-451 on Page 25 in the revised manuscript)

"The present manuscript is a very busy work with many analytical and theoretical considerations, but no experimental proof of the proposed materials concept."

Our Response:

We appreciate the reviewer's recognition of the extensive analytical and theoretical work included in our manuscript. Importantly, we have provided comprehensive experimental evidence throughout the paper to validate the formation and function of the tensegrity structures. For example, we include:

Microscopic Characterization (Fig. 2): SEM and AFM images that clearly show the interlocked crystal structures within the hydrogel.

Internal Stress Measurements (Fig. 3): Fluorescence-based assays (using GFP as a force probe) that directly indicate the development of internal tension due to crystal growth.

Mechanical Testing (Fig. 4): Tensile and compressive tests that demonstrate the unique mechanical properties (including a tensile-to-compressive modulus ratio of up to 13).

Molecular Dynamics Simulations (Fig. 6): Computational models that support our experimental observations and provide mechanistic insight into the structure–property relationships.

We believe that this combination of experimental and theoretical evidence robustly supports the proposed materials concept and its novelty. We also emphasized this point in the revised manuscript. (See line 470-478 on Page 25-26 in the revised manuscript)

"There is no proof that the material can be imprinted with anisotropic mechanical properties by applying external stress. Therefore, these claims cannot be held up and this weakens the manuscript."

Our Response:

We apologize if any statements in the manuscript led to the impression that our TG hydrogels were designed to exhibit externally imprinted anisotropic mechanical properties. In our work, the hydrogels do not display traditional anisotropy—i.e., different properties along different spatial axes—but rather exhibit bimodular (nonreciprocal) behavior. Bimodular behavior refers to the difference in mechanical

response when forces are applied in opposite directions along the same axis (for example, differing tensile and compressive moduli). This distinction is critical and is supported by our experimental data as well as by literature (e.g., J. Compos. Mater. 1976, 10, 342; AIAA J. 1977, 15, 16; J. Mech. Sci. Technol. 2010, 24, 1845; J. Mech. Phys. Solids 2014, 73, 183). We have now highlighted it in the manuscript to more clearly define and emphasize the concept of bimodularity versus traditional anisotropy, which we believe resolves this concern. (See line 71-76 on Page 4 in the revised manuscript)

Revision:

In summary, we appreciate the reviewer's insights and have revised the manuscript accordingly to:

(1) Emphasize that the novelty of our work lies not solely in the chemistry, but in achieving a unique combination of mechanical properties via the formation of tensegrity structures. (See line 457-466 on Page 25 in the revised manuscript)

The corresponding revision is also attached below:

“Although the chemistry of dephosphorylation and subsequent crystal growth has been previously reported,^{45,73,74} the in-situ growth of amino acid crystal sticks in this work integrates rigid and soft components into a cohesive structure, leading to the formation of tensegrity architectures. This process not only introduces prestressed conditions in the polymer network but also enables the formation of interlocked crystal elements that reinforce the hydrogel without sacrificing its water content. It is this special combination of structural design and mechanochemical interplay that results in a unique set of mechanical properties that has not been achieved in hydrogels lacking tensegrity architectures.”

(2) Clarify that the crystal growth process naturally induces a sharp transition in mechanical properties through internal structural evolution—not through externally imposed anisotropy. (See line 449-451 on Page 25 in the revised manuscript)

The corresponding revision is also attached below:

“The in-situ crystal growth process naturally induces a sharp transition in mechanical properties through internal structural evolution rather than externally imposed anisotropy.”

(3) Reaffirm that extensive experimental evidence supports the formation and function of the tensegrity structures, which underpin the observed mechanical behavior. (See line 470-478 on Page 25-26 in the revised manuscript)

The corresponding revision is also attached below:

“The experimental evidence, including SEM and AFM images, clearly demonstrates the formation of interlocked crystal structures within the hydrogel, while fluorescence-based assays directly indicate the development of internal tension due to crystal growth. The presence of tensegrity structures contributes to the observed mechanical behavior, including a tensile-to-compressive modulus ratio of up to 13 in tensile and compressive tests. Molecular dynamics simulations further illustrate how mechanochemistry drives the formation of these tensegrity structures. The simulations reveal that the tension generated within the polymer network during crystal growth propagates long-distance stresses, enhancing the mechanical integrity and enabling a bimodulus response.”

(4) Clearly differentiate between bimodular (nonreciprocal) behavior and traditional anisotropy to prevent any further misunderstandings. (See line 71-76 on Page 4 in the revised manuscript)

The corresponding highlight is also attached below:

“These materials display anisotropic mechanical responses along different axes of applied forces, in accordance with their anisotropic structures. In contrast, tensegrity structures demonstrate fundamentally different mechanical properties, exhibiting bimodulus or nonreciprocal mechanical responses—where the behavior varies under forces applied in opposite directions along the same axis.⁵⁰⁻⁵³”

We trust that these revisions and clarifications adequately address the reviewer’s concerns and highlight the true novelty and impact of our work. We thank the reviewer

again for their constructive feedback.

Modelling is useless without experimental proof. The tensegrity structures should be resulting in an anisotropic stiffness as is the case for bones. Thus, all the theoretical statements and fluorescence probes do not show this anisotropy. Further, none of the freeze-dried samples shows any anisotropic growth as always stated in the manuscript. The orienting of needle crystals in hydrogels at extreme mechanical deformation is expected and well known, but does not require any directed crystal growth and works with any hydrogel system. At the current state, the manuscript is reporting on a novel concept to use the known ALP-method with an extremely expensive system to obtain hydrogels that are not particularly well performing compared to other stiff hydrogels.

Response:

We thank the reviewer for the comments. As in our response to previous concerns, we would like to emphasize that, in addition to the high strength-to-weight ratio, our work focuses on reporting the bimodulus property of hydrogels with prestressed tensegrity structures, which is distinct from traditional anisotropy. The tensegrity structure does NOT result in anisotropic stiffness, and the theoretical statements and fluorescence probes in our study were not intended to demonstrate anisotropic structures or mechanical properties. We apologize for any confusion caused by the statement in the caption of Fig. 1, which reads, “ALP was covalently integrated into the hydrogel network to induce in-situ anisotropic growth of amino acid crystal sticks along the axis direction of pre-loaded crystal nuclei.” This could have led to a misunderstanding about the anisotropic alignment of crystal sticks. The intended meaning was that the amino acid crystal sticks grow along the axis of pre-loaded crystal nuclei, not that they align anisotropically within the hydrogel. The crystal sticks exert tension on the elastic network, while the network applies compression to the crystal sticks, forming a tensegrity structure. Now we have revised the statement in the figure caption to clarify this point. (See line 123-124 on Page 7 in the revised manuscript)

We appreciate the reviewer’s comment regarding the integration of modelling with

experimental data. In our study, the MD simulations are not intended to replace experimental evidence but rather to serve as a complementary tool, offering microscopic insight into the mechanochemical interactions that underlie the bimodular behavior of our hydrogels. Specifically, the simulations revealed that tensile stretching further extends the prestressed polymer chains, thereby enhancing the tensile Young's modulus, while compression causes the soft polymer network to extrude, releasing internal stress and reducing the compressive modulus (Fig. 6g-h). Moreover, the distinct stress distributions observed at individual crosslinking sites confirm the tensile state of the polymer network under both loading conditions (F_T in Fig. 6g-h). Our experimental work—including extensive structural characterization, fluorescence-based internal stress measurements, and mechanical testing—demonstrates the formation and function of the tensegrity structures. Together, these experiments validate the concepts derived from our simulations and provide a comprehensive understanding of the structure–property relationship.

We acknowledge the reviewer's observation regarding the orientation of needle crystals under extreme deformation. Our manuscript does describe the reorientation of needle-like crystals during large deformations; however, we did not intend to claim that such reorientation arises from a directed or externally imprinted crystal growth process. Rather, it is an inherent consequence of the internal prestress and the reorganization of the tensegrity structure under load. In our revised manuscript, we have clarified that the observed needle crystal orientation during deformation is a response to mechanical stress and is not indicative of a deliberately engineered anisotropic structure. (See line 414-417 on Page 22-23 in the revised manuscript)

We appreciate the reviewer's comment regarding the cost and performance aspects of the ALP method. While it is true that the ALP-based approach represents a more complex (and potentially higher-cost) system compared to some conventional methods, the primary novelty of our work lies in the integration of in-situ crystal growth with a prestressed polymer network to generate tensegrity architectures. This design achieves

a unique combination of high stiffness, excellent fracture toughness, and high water content, along with pronounced bimodular mechanical behavior—properties that are not simultaneously realized in conventional hydrogels. Moreover, we discuss in the revised manuscript how the tensegrity strategy can be extended to alternative, potentially more cost-effective systems (e.g., using inorganic salt crystal nucleation via hydrothermal processes or catalytic reduction), thereby broadening the scope and practical applicability of our approach. (See line 496-499 on Page 27 in the revised manuscript)

Revision:

“ALP was covalently integrated into the hydrogel network to induce in-situ growth of amino acid crystal sticks.”

“Additionally, the internal stress induced the rearrangement of crystal sticks under force, as confirmed by MD simulations and SEM imaging (Supplementary Figs. 38-39). This rearrangement contributed to the distinct mechanical response of the hydrogels under large deformations.”

“Following these rules, the tensegrity can also be extended to other hydrogel system. For example, by inducing the in-situ crystal growth of inorganic salt at large aspect ratios via hydrothermal process or catalytic reduction, tensegrity in hydrogels can be achieved without bio-synthesis steps.”

There are - besides the typos - some other points that should be addressed:

What is the crystal density? I do not understand the unit. Density is something like g/cm³ or vol/vol, but never a 2D unit. It should be provided, what the volume or the weight content of the tyrosine crystals is within the matrix.

Response:

We thank the reviewer for the comments. The crystal density in the original manuscript was determined by analyzing the number of crystals per unit area observed in the

hydrogels via SEM imaging. We acknowledge that the 2D analysis may not accurately reflect the true crystal density. In response to the reviewer's comments, we have now included the weight content of tyrosine crystals within the matrix in Fig. 5b. Additionally, we have thoroughly reviewed and corrected any typographical errors throughout the manuscript. (See line 350-354 on Page 19 and Figure 5 on Page 21 in the revised manuscript; See line 81-86 on Page 3 in the revised Supplementary Information)

Revision:

“The crystal showed a 50-fold increase of weight content, with sizes growing from less than 5 μm to over 20 μm (Fig. 5a-c and Supplementary Fig. 32). After 6 hours, crystal growth along the long axis of crystal nuclei became slow, likely due to strain hardening effect of the polymer network, which constrains the further growth of crystals, but the weight content continued to rise (Fig. 5b-c).”

“Weight content of crystals

For the weight content of crystals at different time points, the hydrogels were initially weighed, with the wet weight recorded as W_1 , and then lyophilized, with the dry weight recorded as W_2 . The weight of the polymers (W_0) in the hydrogels was estimated based on the water content and the initial volume before crystal growth. The weight content of crystals was then calculated as $(W_2 - W_0)/W_1 \times 100\%$.”

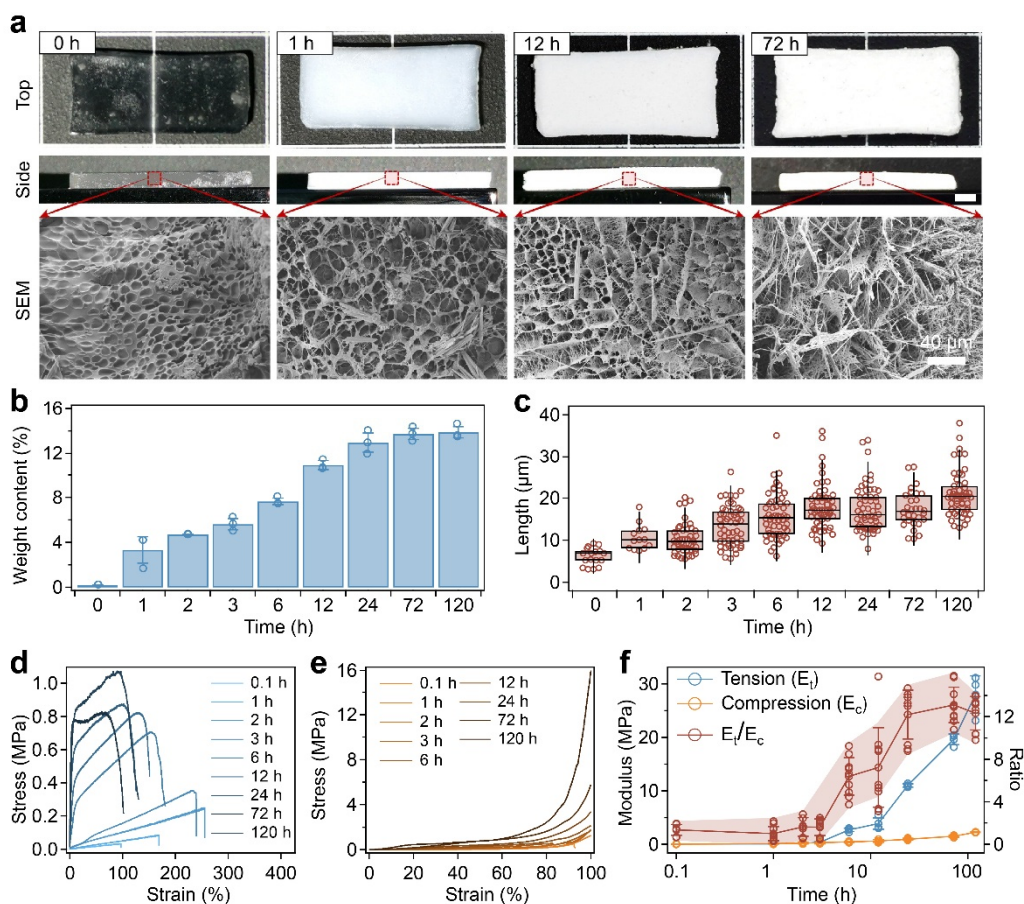


Figure 5 Kinetics of tensegrity structure formation. **a**, Optical (top) and SEM (bottom) images of band-shaped TG hydrogel after in-situ crystal growth for different times (0, 1, 12 and 72 h). The scale bar of optical images is 5 mm. **b**, **c**, Weight content (**b**) and length (**c**) of crystals in TG hydrogels after in-situ crystal growth for different times (0, 1, 2, 3, 6, 12, 24, 72 and 120 h). **d**, **e**, Typical stress–strain curves of TG hydrogels under tension (**d**) and compression (**e**) after in-situ crystal growth for different times (0.1, 1, 2, 3, 6, 12, 24, 72 and 120 h). **f**, Summarized tensile and compressive Young's moduli, and ratios of tensile to compressive moduli (E_t/E_c) of TG hydrogels after in-situ crystal growth for different times (0.1, 1, 2, 3, 6, 12, 24, 72 and 120 h). Values represent the mean and standard deviation ($n \geq 3$).

Ratio E_t/E_c is pointless, because the compression modulus is varying along the curve.

Response:

We thank the reviewer for the comments. The ratio of tensile to compressive moduli

(E_t/E_c) has been widely used in the literature to characterize the bimodulus properties of materials (J. Mech. Phys. Solids 2014, 73, 183; Appl. Math. Model. 2019, 66, 41; Comput. Mech. 2019, 63, 335–363; Comput. Struct. 2020, 229, 106176). In this study, we adopted this ratio to describe the bimodulus behavior of TG hydrogels. While the compressive modulus varies along the curves, as noted by the reviewer, we calculated the ratio using the compressive modulus at a fixed strain range (0-20%). This approach allows for a direct comparison of the ratios across different hydrogels and time points. Additionally, we have cited further references discussing the tensile-to-compressive modulus ratio in the revised manuscript. (See reference Nos. 53 and 65-67 on Page 42-44, line 310-312 on Page 16 in the revised manuscript)

Revision:

“The ratio of tensile to compressive moduli (E_t/E_c)^{53,65-67} reached as high as 13 at 20 mM bis-acrylamide concentrations, a feature not observed in ALP/PAAm hydrogels.”

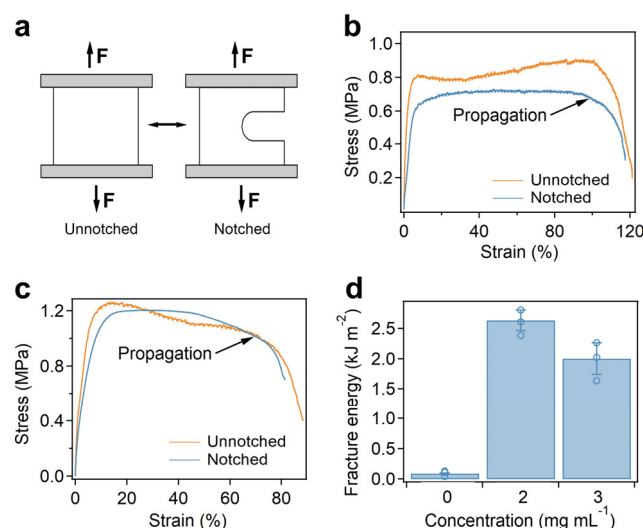
What are the values of the fracture strain of the notched samples and how does such a curve look?

Response:

We thank the reviewer for the comments. We have provided the stress-strain curves for notched samples in Supplementary Fig. 18. The fracture strains of the notched TG hydrogels were similar to those of the unnotched samples, indicating their crack insensitivity. As a result, the TG hydrogels exhibited relatively high fracture energies, reaching up to 2600 J m⁻². The new comment have been included in the revised manuscript. (See line 277-279 on Page 15 in the revised manuscript; See Supplementary Figure 17 on Page 15 in the revised Supplementary Information)

Revision:

“The notched TG hydrogels exhibited fracture strains similar to those of the unnotched samples, leading to relatively high fracture energies.”



Supplementary Figure 17. Fracture energy determination of TG hydrogels. **a**, Schematic illustration of stretching unnotched and notched hydrogel samples. **b**, **c**, Typical stress–strain curves of notched and unnotched TG hydrogels under tension at crystal nucleus concentrations of 2 (**b**) and 3 (**c**) mg mL^{-1} . The arrows indicate the points where the crack starts to propagate in the notched samples. The width of the band-shaped hydrogel was 8 mm, the crack length of the notched hydrogel was 1.6 mm, and the initial distance between the two clamps was set at 3 mm. **d**, Fracture energies of TG hydrogels at different concentrations of crystal nuclei (0, 2 and 3 mg mL^{-1}). Values represent the mean and standard deviation ($n = 3$).

Figure 4 is misleading, because the authors have used arbitrary literature to make their material look exceptional. There are mineralized hydrogels that have more than 300 MPa tensile stiffness, more than 70 wt% water and more than 2000 J/m^2 and very high tensile strength. There should be a thorough literature study for this figure.

Response:

We appreciate the reviewer’s insightful comments. To avoid the misleading, we have removed Fig. 4g and incorporated more representative literatures into the Supplementary Table 1, including additional references on mineralized hydrogels and glassy-state hydrogels, as suggested. Notably, some mineralized hydrogels exhibit a higher modulus than TG hydrogels while maintaining relatively high water content

(*Chem. Mater.* 2021, 33, 8312; *J. Mater. Sci.* 2021, 56, 15299). Similarly, glassy-state hydrogels also demonstrate a combination of high modulus and high water content (*Chem. Eng. J.* 2019, 362, 325; *Chem. Mater.* 2019, 31, 1430). The relevant data can be found in revised Supplementary Table 1. (See Supplementary Table 1 on Page 31-32 in the revised Supplementary Information)

Revision:

Supplementary Table 1. Summary of water contents and Young's moduli of TG hydrogels and other reported stiff hydrogels.

Classification	Sample code	Water content (%)	Young's modulus (MPa)	Reference
This work	TG hydrogel	80-82.5	11-31	--
Single-network hydrogel ①	PIC hydrogel	42.5-64.5	0.03-7.9	<i>Adv. Mater.</i> 27, 2722-2727 (2015). ¹
	DMAA-MAAc hydrogel	50-70	0.01-8	<i>Adv. Mater.</i> 27, 6899-6905 (2015). ²
Double-network hydrogel ②	alginate–polyacrylamide hydrogel	84	0.11	<i>Nature</i> 489, 133–136 (2012). ³
	C4-G20-Px hydrogel	51-81	0.03-2.47	<i>Sci. Adv.</i> 5, eaau3442 (2019). ⁴
Dual-crosslinked hydrogel ③	HN-PH ₆ hydrogel	80-92	0.1-0.3	<i>Sci. Adv.</i> 6, eaaz9531 (2020). ⁵
	p-Pep/Cu ²⁺ hydrogel	87-93	0.1-0.14	<i>Nat. Commun.</i> 14, 2583 (2023). ⁶
Mineralized hydrogel ④	PAAm-l-MBAm (ALP-induced)	59-70	6.7-103	<i>Nature</i> 543(7645), 407-410 (2017). ⁷
	Mineralized star-P(EO-stat-PO) hydrogel	50-75	2.1-16.1	<i>Mater. Sci. Eng. C Mater. Biol. Appl.</i> 75, 471-477 (2017). ⁸
	PDMA-co-AA-I-TEG	56-69	280–300	<i>Chem. Mater.</i> 33, 8312-8322 (2021). ⁹
	PAAm-l-MBAm (Urea-induced)	~50	455	<i>J. Mater. Sci.</i> 56, 15299-15312 (2021). ¹⁰
Highly entangled hydrogel ⑤	Highly entangled PAAm	82-91	0.1-0.2	<i>Science</i> 374, 212-216 (2021). ¹¹
	N-DC (FL) ₈ hydrogel	72-80	0.53-0.7	<i>Nature</i> 618, 740–747 (2023). ¹²

Hierarchical structured hydrogel ⑥	HA-PVA hydrogel	70	2.5	<i>Nature</i> 590, 594–599 (2021). ¹³
	FC-EtFe hydrogel	88-90	1.4-2.0	<i>Sci. Adv.</i> 9, eadf7075 (2023). ¹⁴
Glassy-state hydrogels ⑦	P(PA-co-AAm) hydrogel	50–60	150–280	<i>Chem. Eng. J.</i> 362, 325-338 (2019). ¹⁵
	P(MAAm-co-MAAc) hydrogel	50-70	2.3–217.3	<i>Chem. Mater.</i> 31, 1430-1440 (2019). ¹⁶

The manuscript is very difficult to read, because major data are in the supplements and thus the manuscript cannot be understood without frequently flipping to the SI. It would be better to reduce the non-proven over claims (like the cartoons) and present this study as full paper containing all necessary data in the main text.

Response:

We thank the reviewer for the constructive feedback. To the best of our knowledge, our work is the first report of engineered hydrogels that incorporate tensegrity structures. Because this is a novel concept, it is essential that we provide comprehensive experimental evidence to support our findings. We recognize that our original manuscript may not have provided sufficient background on the unique concepts of tensegrity and bimodularity, which—unlike traditional anisotropy—refers to different mechanical responses under tension versus compression along the same axis. This may have led to some confusion with respect to anisotropy. In our revised manuscript, we have added a more detailed introduction to these concepts to clarify that our hydrogels exhibit bimodular behavior rather than conventional directional anisotropy. (See line 71-76 on Page 4 in the revised manuscript)

Regarding the comment that major data are relegated to the Supplementary Information, we would like to emphasize that all critical results supporting the design, formation, and evolution of the tensegrity structures, along with the corresponding MD simulations, are now fully integrated into the main text. The cartoons in these figures are meant solely to aid in visualizing complex concepts, not to make unproven claims. For example, Fig. 1 schematically illustrates our design of tensegrity structures, while Fig.

3 and Fig. 6 present the experimental settings, internal stress measurements, and simulation snapshots that underpin our conclusions. We have revised the corresponding captions to explicitly state their role in aiding understanding. (See line 241-244 on Page 13 and line 249-253 on Page 14 in the revised manuscript)

We believe that these revisions will enhance the manuscript's clarity and accessibility, allowing readers to fully appreciate the novelty of our tensegrity-based hydrogel system. We thank the reviewer again for the opportunity to improve our manuscript.

Revision:

“These materials display anisotropic mechanical responses along different axes of applied forces, in accordance with their anisotropic structures. In contrast, tensegrity structures demonstrate fundamentally different mechanical properties, exhibiting bimodulus or nonreciprocal mechanical responses—where the behavior varies under forces applied in opposite directions along the same axis.⁵⁰⁻⁵³”

“a, Schematic illustration depicting the experimental setup for probing internal tensional forces using green fluorescent protein (GFP) as a fluorescent force probe. GFP unfolds due to polymer stretching driven by crystal growth, resulting in fluorescence diminishing.”

“e, Schematic illustration depicting the experimental setup for visualizing the deformation field of TG hydrogels under applied strain. The movements of green fluorescent microspheres, fixed to the hydrogel network, were recorded before and after crystal growth under an applied strain ($\sim 170\ \mu\text{m}$) using a microneedle and analyzed.”

Reviewer #3 (Remarks to the Author):

This study presents a novel approach for the fabrication of a tensegrity-structured hydrogel through the induction of amino acid crystal. The authors demonstrate that this method exhibits a certain degree of versatility and can be applied to other hydrogel systems. This work offers a breakthrough solution for the elusive combination of high stiffness, strength, water content, and bimodal mechanical response. This innovation has the potential to revolutionize the field of biomimetic materials and may serve as a classic example in the fabrication of gel materials. The manuscript is well-structured, with clear writing, comprehensive data presentation, and high-quality graphical content. It deserves publication in this journal. Other specific comments and suggestions are as follows:

General response:

We thank the reviewer for the positive comments. In order to address her/his concerns, we have conducted a substantial revision with new data, analyses and discussions. The detailed point-by-point response to each of her/his concerns is attached below. The changes to the original submission are highlighted in blue in the revised manuscript and Supplementary Information.

1.Challenges of method scalability and technical barriers. The designed hydrogel materials exhibit remarkable properties, including high water content, stiffness, and toughness. However, the preparation method is relatively complex, particularly the bio-synthesis step, which requires long processing times and involves a high technical barrier. The potential for broader adoption of this interdisciplinary approach, or how to engage a wider community of hydrogel researchers, is an issue worth considering. The authors might contemplate discussing the scalability of this method, exploring how it could be simplified or made more accessible to a broader audience, especially considering the involvement of bio-synthesis.

Response:

We thank the reviewer for the comments. The tensegrity strategy presented in this work can be extended to other systems by following several key principles for building

tensegrity structures. First, elements with different stiffnesses are required, where the stiff elements serve as the interlocked rigid skeleton and the soft elements function as soft linkers. Second, the stiff elements with a large aspect ratio should have a disordered orientation to facilitate their mechanical interlocking at low mass contents. Third, the soft linkers or elastic soft network must be prestressed to enable immediate stress transfer upon mechanical loading, maintaining structural integrity even under significant strain.

Following these rules, the tensegrity can also be extended to other hydrogel system. For example, by introducing the crystal nuclei of inorganic salts during preparation of hydrogels, the in-situ crystal growth of inorganic salt can be achieved via hydrothermal process or catalytic reduction. Through controlling the large aspect ratio, tensegrity in hydrogels can be achieved without bio-synthesis steps. Moreover, tensegrity can also be achieved in other soft materials such as dry elastomers. For example, by introducing a soft polymer network filled with a precursor of a stiff, rigid anisotropic polymer, the tension generated by the stiff polymer can induce tensegrity when the crosslinking of the stiff polymer is triggered within the soft polymer network under stretching. The new discussions have been included in the revised manuscript. (See line 489-503 on Page 26-27 in the revised manuscript)

Revision:

“To achieve a tensegrity structure inside soft materials, several key factors need to be designed rationally. First, elements with different stiffnesses are required, where the stiff elements serve as the interlocked rigid skeleton and the soft elements function as soft linkers. Second, the stiff elements with a large aspect ratio should have a disordered orientation to facilitate their mechanical interlocking at low mass contents. Third, the soft linkers or elastic soft network must be prestressed to enable immediate stress transfer upon mechanical loading, maintaining structural integrity even under significant strain. Following these rules, the tensegrity can also be extended to other hydrogel system. For example, by induce the in-situ crystal growth of inorganic salt at

large aspect ratios via hydrothermal process or catalytic reduction, tensegrity in hydrogels can be achieved without bio-synthesis steps. Moreover, tensegrity can also be achieved in other soft materials such as dry elastomers. For example, by introducing a soft polymer network filled with a precursor of a stiff, rigid anisotropic polymer, the tension generated by the stiff polymer can induce tensegrity when the crosslinking of the stiff polymer is triggered within the soft polymer network under stretching.”

2.Comprehensive evaluation of the strengths and weaknesses of gel mechanical properties. Compared to glassy-state hydrogels (Chem. Mater. 2019, 31 (4)); Chem. Eng. J. 2023. 457 (141280)) the hydrogel presented in this study exhibits notable advantages, including high water content (>80%) and a bimodal mechanical response. In the Introduction or Discussion sections, the authors should provide further explanations of the significance and potential applications of these two key advantages. A clearer rationale for why these properties are desirable, along with specific contexts in which these hydrogels could be applied, would strengthen the application relevance of the study.

Response:

We thank the reviewer for the comments. Following the reviewer’s suggestion, we have discussed the significance and potential applications of bimodulus property and high water content. We anticipate that the bimodulus property and high water content in TG hydrogels could have significant applications in tissue engineering. For example, in cells, the tensegrity structure supports the resilience and adaptability of the cytoskeleton and extracellular matrix (J. Cell Sci. 1993, 104 (Pt 3), 613-627; Osteoarthr. Cartilage 1999, 7, 81-94; Prog. Biophys. Mol. Biol. 2008, 97, 163-179; Cureus 2020, 12, e7895), both of which exhibit nonreciprocal mechanical responses crucial for cellular functions. The tensegrity strategy presented in this work offers a general approach for fabricating bimodular synthetic hydrogels, which will undoubtedly benefit the development and application of biomaterials that more closely mimic the mechanical properties of cytoskeleton and extracellular matrix. We have included the new discussions and cited the references mentioned by the reviewer in the revised manuscript. (See line 50-57 on

Revision:

“These unique properties have a substantial impact on the mechanical behavior of biological systems. For example, in cells, the tensegrity structure supports the resilience and adaptability of the cytoskeleton¹⁸⁻²⁰ and extracellular matrix^{21,22}, both of which exhibit nonreciprocal mechanical responses crucial for cellular functions. Hence, bio-inspired design of hydrogels incorporating tensegrity structures is a promising strategy for creating robust, adaptive materials that maintaining a high water content, which are critical for biological functions and biomimetic applications, such as tissue engineering.²³⁻²⁷”

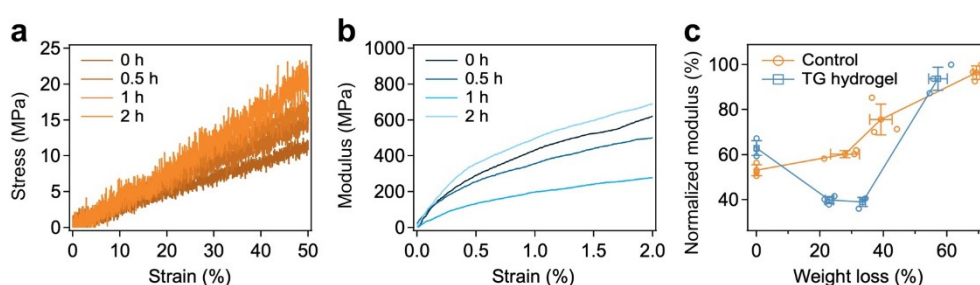
3. Additional evidence of key role of the prestressed polymer matrix at the bulk level. As discussed throughout the manuscript, the prestressed polymer matrix plays a critical role in the tensegrity structures and contributes to the excellent mechanical properties. While the tension force on the polymers at the nano/microscale has been thoroughly evaluated, it would be beneficial to provide additional evidence for the role of the tensioned polymer network at the bulk level, such as the release of tension force through moderate dehydration.

Response:

We thank the reviewer for the comments. Following the reviewer’s comment, we have studied the effect of the releasing tension force on the mechanical properties of TG hydrogels by moderate dehydration. As shown in Supplementary Fig. 25, the tensile modulus of TG hydrogels first decreased and then increased during the dehydration process, in contrast to the continuous decrease observed in PAAm/ALP hydrogels. This indicates that the release of internal stress on polymer networks initially leads to a decrease in the mechanical strength of TG hydrogels despite the increase in solid content. These results highlight the crucial role of tensioned polymer network in tensegrity structures at the bulk level. The new data and discussion have been included in the revised manuscript. (See line 303-307 on Page 16 in the revised manuscript; See

Revision:

“Moreover, the tensile modulus of TG hydrogels initially decreased and then increased upon releasing tensile force through moderate dehydration, in contrast to the continuous increase observed in PAAm/ALP hydrogels (Supplementary Fig. 25). This behavior further highlights the crucial role of stress on polymers in tensegrity structures and contributes to the high Young’s modulus.”



Supplementary Figure 25. Mechanical properties of TG and control (ALP/PAAm) hydrogels during dehydration over time. **a**, Typical stress–strain curves of control (ALP/PAAm) hydrogels under tension after dehydration for different times (0, 0.5, 1, and 2 hours). **b**, Typical stress–strain curves of TG hydrogels under tension after dehydration for different times (0, 0.5, 1, and 2 hours). **c**, Summary of the modulus of TG and control (ALP/PAAm) hydrogels over time during dehydration. The moduli of the hydrogels after dehydration for 2 h were set as 100%. Values in **c** and **d** represent the mean and standard deviation ($n = 3$).

4. Further processing of the tensegrity structures hydrogels. As described in the manuscript, the tensegrity structure was fabricated through in-situ crystal growth. Since the ALP enzyme is covalently linked to the hydrogel network, it would be valuable to explore methods for spatially confining the tensegrity structures within the hydrogel. This could potentially serve as an additional processing technique for these hydrogels.

Response:

We thank the reviewer for their insightful comments. In response, we have explored

methods to further process the tensegrity structures in hydrogels. By leveraging the kinetic process of crystal growth, spatial control of tensegrity structures can be achieved through modulation of the SC-ALP distribution. As shown in Supplementary Fig. S35, a precursor solution containing SC-ALP was used as ink to print hydrogel patterns via a DLP 3D printer, with the remaining areas filled using PAAm hydrogels. After in situ crystal growth for 1 hour, complex TG hydrogel patterns, such as an “NJU” logo and multi-layer grids, were successfully produced. The new data and experimental details have been included in the revised manuscript. (See line 376-380 on Page 20 in the revised manuscript; See line 89-101 on Page 4 and Supplementary Figure 35 on Page 28 in the revised Supplementary Information)

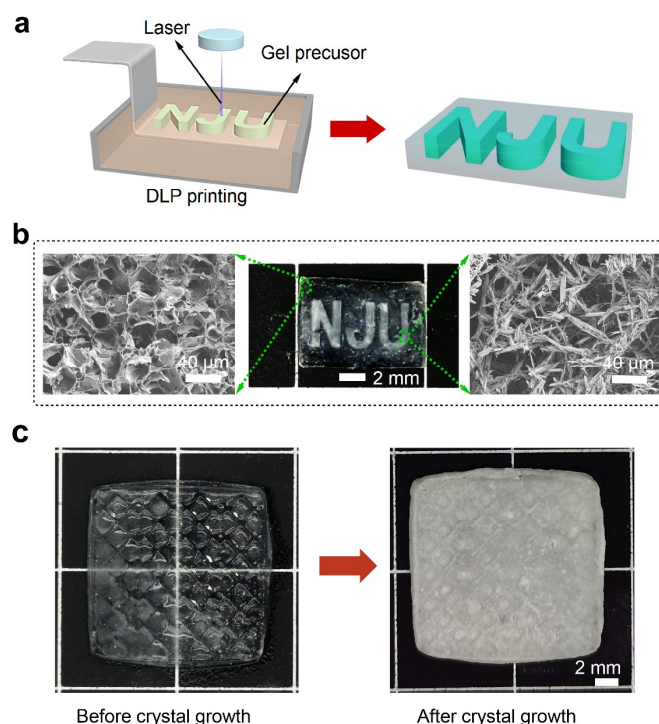
Revision:

“Notably, patterned TG hydrogels can be fabricated using digital light processing (DLP) 3D printing⁷⁵, enabling spatially controlled crystal growth. This method successfully produced complex patterns, such as an “NJU” logo and multi-layer grids, within the hydrogels (Supplementary Fig. 35), highlighting the potential for constructing intricate TG hydrogel structures.”

“Digital Light Processing (DLP) 3D printing of TG hydrogels

For the DLP 3D printing of hydrogels, acrylamide, bis-acrylamide, acrylate-SpyTag, SC-ALP, and LAP were dissolved in a saturated tyrosine solution at concentrations of 1000, 20, 0.1, 0.05, and 0.85 mM, respectively. The resulting solution was stored at room temperature for 10 minutes to allow the reaction between SpyCatcher and SpyTag. Crystal nuclei (2 mg mL⁻¹) were then added to the solution and dispersed homogeneously using a vortex oscillator (VORTEX 2, IKA, Germany). The prepared precursor solution was used as ink to print hydrogel patterns or multi-layer grids using a custom-made DLP 3D printer equipped with a CUV4704 light source (Crystal-Optech, China). The remaining areas were filled with PAAm hydrogels. The printed hydrogel patterns or multi-layer grids were subsequently immersed in a solution for in situ crystal growth. After incubation at 37 °C for 1 hour, the patterned TG hydrogels were

successfully obtained.”



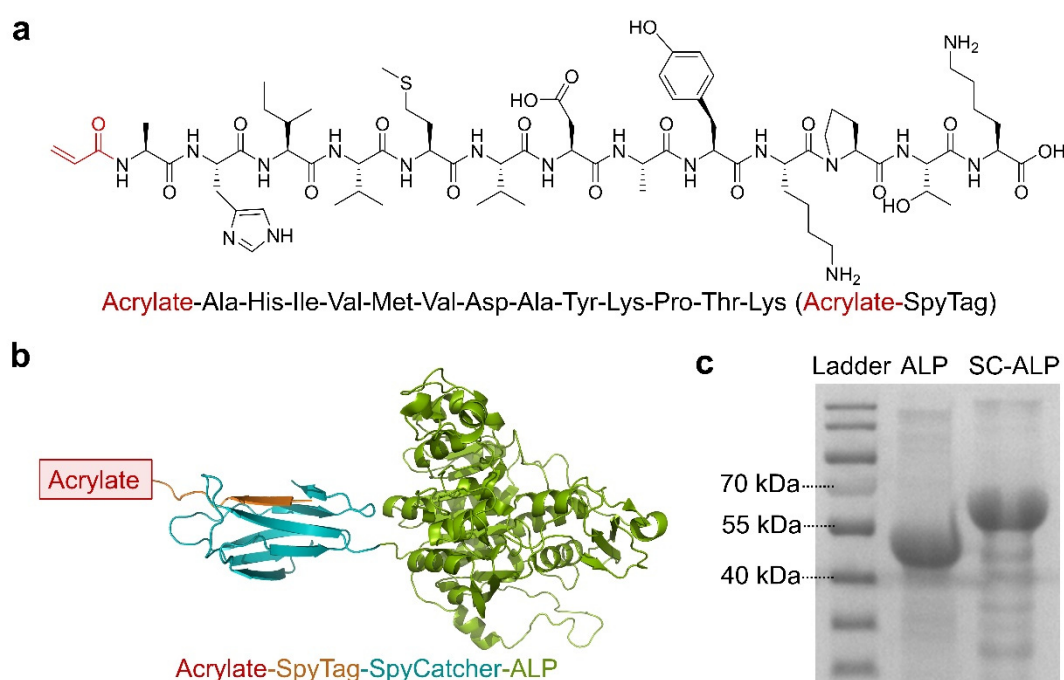
Supplementary Figure 35. Fabricating patterned TG hydrogels using DLP 3D printing. **a**, Schematic of the experimental setup for the fabrication of patterned TG hydrogels using DLP 3D printing. A hydrogel precursor containing SC-ALP was used to print a pattern of "NJU" under ultraviolet laser, followed by using a precursor without SC-ALP to print the remaining areas. The resulting hydrogel was used to complete the in-situ crystal growth. **b**, Photograph and SEM images of patterned TG hydrogels fabricated using DLP 3D printing. **c**, TG hydrogel with multiple-layer grids fabricated using DLP 3D printing. The optical images correspond to the hydrogel before (left) and after (right) in-situ crystal growth.

5. Some minor issues. (1) According to the figure S2, the group of “-NH-” at the left terminal is labeled in red. As I am concerned, it might come from the carboxyl group from alanine next to acrylate, in which case it should be labeled in black. (2) It’s recommended to include a flow chart for the preparation process of hydrogels to help with the quick understanding of the formation of tensegrity structured hydrogels.

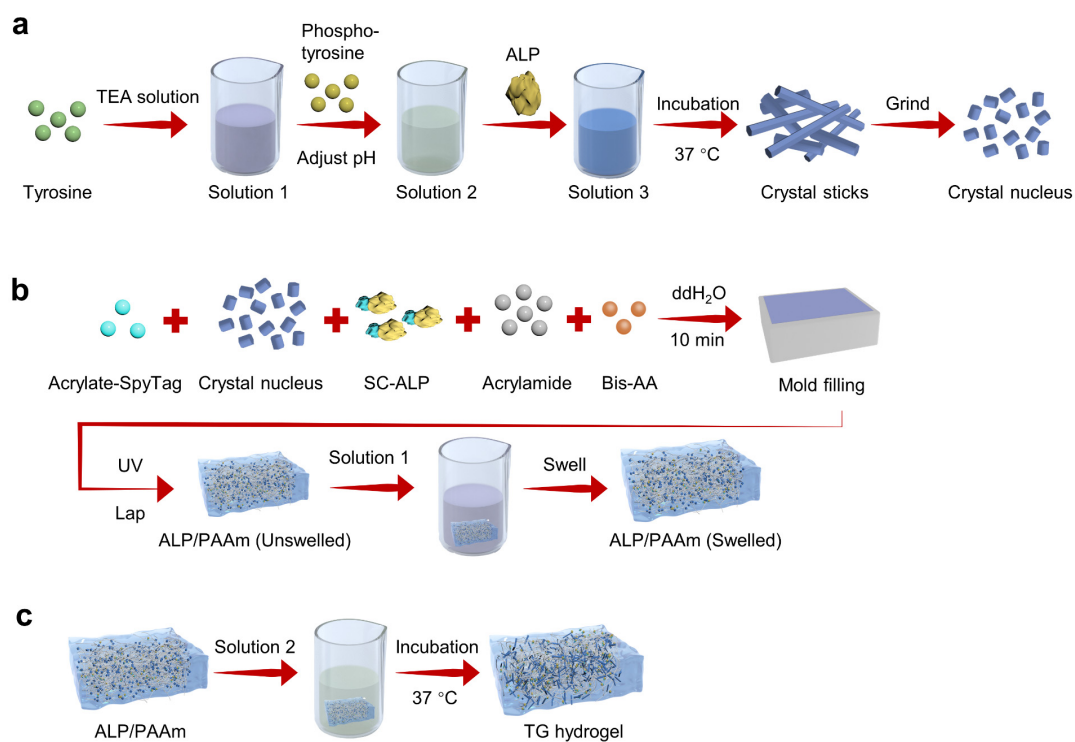
Response:

We thank the reviewer for the comments. Following the reviewer's comments, we have revised the manuscript with these minor issues. (1) The group of “-NH-” at the left terminal of Supplementary Fig. 2a has been labeled in black now. (2) We have added a flow chart (Supplementary Fig. 40) to clarify the preparation process of hydrogels following the reviewer's suggestion. (See Supplementary Figure 2 on Page 5 and Supplementary Figure 40 on Page 31 in the revised Supplementary Information)

Revision:



Supplementary Figure 2. Structure of acrylate-SpyTag and SpyCatcher-ALP (SC-ALP). **a**, Chemical structure of acrylate-SpyTag. **b**, Schematic representation of the conjugation between SC-ALP and acrylate-SpyTag. **c**, SDS-PAGE of ALP and SC-ALP.



Supplementary Figure 40. Flow chart for the preparation of TG hydrogels. **a**, Preparation of crystal sticks and nucleus. **b**, Preparation of the ALP/PAAm hydrogel. **c**, In situ crystal growth of the ALP/PAAm hydrogel to prepare the TG hydrogel.

Reviewer #4 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

General response:

We thank the reviewer for his/her time and insightful comments. In order to address her/his concerns, we have conducted a substantial revision with new data, analyses and discussions. The detailed point-by-point response to each of her/his concerns is attached above. The changes to the original submission are highlighted in blue in the revised manuscript and Supplementary Information.

Point-by-point response to the reviewers' comments

Reviewer #1 (Remarks to the Author):

The authors have adequately addressed all our questions.

Response: We sincerely appreciate the reviewer's comments and the time dedicated to evaluating our manuscript.

Reviewer #2 (Remarks to the Author):

The authors have clarified to manuscript according to the reviewer suggestions and the manuscript should be published after some minor additions. Tensegrity is a structural concept with a rather unclear concept for applications. The authors should give some important examples of biotensegrity in nature and also some application possibilities, where this mechanical behaviour is of importance.

The samples clearly show plastic deformation indicated by the stress-strain-curve measured with a tensile tester. These results can hardly be compared to measurements with a dynamic mechanical analysis, which is often used for such tests. Thus, the samples are not more elastic nor stretchable than comparable materials in literature. However, the strong toughness of the notched sample should be highlighted, because this is a rare feature in stiff hydrogels.

On a minor note, the enzyme used for comparable mineralized hydrogels in supplementary Table 1 is not urea, but urease.

General response: We sincerely appreciate the reviewer's positive comments and the time and effort dedicated to evaluating our manuscript. We have carefully considered all the comments and revised the manuscript accordingly. A detailed point-by-point response to each of the reviewer's concerns is provided below. All changes to the original submission are highlighted in blue in the revised manuscript.

Below is our detailed response to his/her comments.

"The authors have clarified to manuscript according to the reviewer suggestions and the manuscript should be published after some minor additions. Tensegrity is a

structural concept with a rather unclear concept for applications. The authors should give some important examples of biotensegrity in nature and also some application possibilities, where this mechanical behaviour is of importance."

Response:

We thank the reviewer for the comment. In response to the suggestion, we have incorporated key examples of biotensegrity in nature and expanded the discussion on its potential applications where this mechanical behavior plays a crucial role. At the microscale, a representative tensegrity structure is the cytoskeleton, which consists of rigid microtubules acting as interlocked structural elements and tensile actin filaments serving as flexible linkers. This arrangement forms a resilient framework capable of effectively responding to external mechanical stress. At the macroscale, the fascial and skeletal system of the human body exemplifies biotensegrity, showcasing the body's viscoelastic nature maintained through a balance of compression and tension. Bones function as compression-resistant struts, while soft tissues—such as muscles, tendons, ligaments, and fascia—form a continuous tension network. Together, they create a dynamic and adaptive structure that withstands external forces and facilitates efficient movement (Journal of Mechanics in Medicine and Biology, 2002, 2(03&04): 375-388; Journal of Osteopathic Medicine, 2013, 113(1): 34-52). These examples of biotensegrity have been incorporated into the revised manuscript. (See line 102-107 on Page 5-6 in the revised manuscript)

Moreover, we have discussed the potential applications where this mechanical behavior of tensegrity structures plays a crucial role. The key advantage of tensegrity structures lies in their high strength-to-weight ratio and exceptional mechanical properties. Another feature is their nonreciprocal mechanical responses, which arise from their distinctive configurations. These characteristics are fundamental to the mechanical properties of biological tissues. Inspired by this, the bioinspired design of hydrogels incorporating tensegrity structures offers a promising strategy for developing robust, adaptive materials for biomedical and biomimetic applications. For instance, TG hydrogels can serve as high-water-content yet stiff scaffolds for cell culture and tissue

repair. The rigid components provide structural stiffness for cell adhesion and mechanical cues to induce differentiation, while the low solid content offers ample space for cell migration and proliferation. Furthermore, the nonreciprocal mechanical responses of TG hydrogels make them suitable for applications such as artificial cartilage for tissue repair and flexible skins for soft robots, allowing them to adapt to varying compressive and tensile stresses during movement. We have added the new discussion in the revised manuscript. (See line 418-429 on Page 20 in the revised manuscript)

Revision:

“Besides the cytoskeleton, the fascial and skeletal system of the human body also exemplifies biotensegrity, demonstrating the body's viscoelastic nature maintained by a balance of compression and tension.⁵⁷⁻⁵⁹ Bones function as compression struts, while soft tissues—such as muscles, tendons, ligaments, and fascia—form a continuous tension network. Together, they create a dynamic and resilient structure that withstands external forces and enables efficient movement.”

“These engineered tensegrity architectures offer distinct mechanical advantages, including a high strength-to-weight ratio and nonreciprocal mechanical responses—features fundamental to biological tissues. Inspired by this, the bioinspired design of hydrogels incorporating tensegrity structures offers a promising strategy for developing robust, adaptive materials for biomedical and biomimetic applications. For instance, TG hydrogels can serve as high-water-content yet stiff scaffolds for cell culture and tissue repair. The rigid components provide structural stiffness for cell adhesion and mechanical cues to induce differentiation, while the low solid content offers ample space for cell migration and proliferation. Additionally, the nonreciprocal mechanical responses of TG hydrogels make them suitable for applications such as artificial cartilage for tissue repair and flexible skins for soft robots, allowing them to adapt to varying compressive and tensile stresses during movement.”

"The samples clearly show plastic deformation indicated by the stress-strain-curve measured with a tensile tester. These results can hardly be compared to measurements with a dynamic mechanical analysis, which is often used for such tests. Thus, the samples are not more elastic nor stretchable than comparable materials in literature. However, the strong toughness of the notched sample should be highlighted, because this is a rare feature in stiff hydrogels."

Response:

We thank the reviewer for the thoughtful comments. We agree that the TG hydrogels exhibit plastic deformation, as indicated by the stress-strain curves with a tensile tester, and acknowledge that these results cannot be directly compared to measurements obtained using dynamic mechanical analysis. In response, we have removed the references (Mater. Sci. Eng. C. 75, 471-477 (2017); Nature 543(7645), 407-410 (2017)) where mechanical properties were measured using dynamic mechanical analysis from Supplementary Table 1. (See Supplementary Table 1 on Page 33-34 in the revised Supplementary Information.)

Furthermore, we appreciate the reviewer's suggestion to emphasize the strong toughness of the notched samples. In response, we have incorporated the corresponding discussion in the revised manuscript. (See lines 236–239 on Page 11-12 in the revised manuscript.)

Revision:

“Furthermore, the notched TG hydrogels exhibited fracture strains similar to those of the unnotched samples, leading to relatively high fracture toughness (fracture energy $\sim 2600 \text{ J m}^{-2}$). The high toughness of the notched samples indicates the high crack resistance of TG hydrogels, a rare feature in stiff hydrogels.”

“On a minor note, the enzyme used for comparable mineralized hydrogels in supplementary Table 1 is not urea, but urease.”

Response:

We thank the reviewer for pointing out the mistake. We have corrected the “urea” into “urease” in Supplementary Table 1 in the revised Supplementary Information. (See Supplementary Table 1 on Page 33-34 in the revised Supplementary Information)

Revision:

Supplementary Table 1. Summary of water contents and Young’s moduli of TG hydrogels and other reported stiff hydrogels.

Classification	Sample code	Water content (%)	Young’s modulus (MPa)	Reference
This work	TG hydrogel	80-82.5	11-31	--
Single-network hydrogel ①	PIC hydrogel	42.5-64.5	0.03-7.9	<i>Adv. Mater.</i> 27, 2722-2727 (2015). ¹
	DMAA-MAAc hydrogel	67	14-28	<i>Adv. Mater.</i> 27, 6899-6905 (2015). ²
Double-network hydrogel ②	alginate–polyacrylamide hydrogel	86	0.11	<i>Nature</i> 489, 133–136 (2012). ³
	C4-G20-Px hydrogel	51-81	0.03-2.47	<i>Sci. Adv.</i> 5, eaau3442 (2019). ⁴
Dual-crosslinked hydrogel ③	HN-PH ₆ hydrogel	80.4	0.27	<i>Sci. Adv.</i> 6, eaaz9531 (2020). ⁵
	p-Pep/Cu ²⁺ hydrogel	87	0.1-0.14	<i>Nat. Commun.</i> 14, 2583 (2023). ⁶
Mineralized hydrogel ④	PDMA-co-AA-I-TEG	~54.5	280–300	<i>Chem. Mater.</i> 33, 8312-8322 (2021). ⁷
	PAAm-l-MBAm (Urease-induced)	~50	455	<i>J. Mater. Sci.</i> 56, 15299-15312 (2021). ⁸
Highly entangled hydrogel ⑤	Highly entangled PAAm	88-100	0.1-0.2	<i>Science</i> 374, 212-216 (2021). ⁹
	N-DC (FL) ₈ hydrogel	80	0.53-0.7	<i>Nature</i> 618, 740–747 (2023). ¹⁰
Hierarchical structured hydrogel ⑥	HA-PVA hydrogel	70-95	~0-2.5	<i>Nature</i> 590, 594–599 (2021). ¹¹
	FC-EtFe hydrogel	88-90	1.4-2.0	<i>Sci. Adv.</i> 9, eadf7075 (2023). ¹²
Glassy-state hydrogels ⑦	P(PA-co-AAm) hydrogel	50–60	150–280	<i>Chem. Eng. J.</i> 362, 325-338 (2019). ¹³
	P(MAAm-co-MAAc) hydrogel	50-70	2.3–217.3	<i>Chem. Mater.</i> 31, 1430-1440 (2019). ¹⁴

Reviewer #3 (Remarks to the Author):

The authors have well addressed my concerns. Now the manuscript is suitable for publication in Nature Communications. An acceptance is recommended.

Response: We sincerely appreciate the reviewer's comments and the time dedicated to evaluating our manuscript.

Reviewer #4 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Response: We sincerely appreciate the reviewer's comments and the time dedicated to evaluating our manuscript.